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The cardioprotective effect of exogenous sphingosine-1-phosphate requires the activation of endogenous sphingosine-1-phosphate via the sphingosine kinase 1

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Purpose: Exogenous administration of sphingosine-1-phosphate (S1P) alone, or as part of high density lipoprotein, protects against myocardial infarction. S1P-induced cardioprotection targets the inhibition of the mitochondrial permeability transition pore via mechanisms that remain unclear.

In the cell, the endogenous production of S1P from sphingosine is dependent on the activation of sphingosine kinases (SphK) 1 and 2. These two kinases play a role in cardioprotection against ischemia-reperfusion (IR) injury. Therefore, we hypothesised that the cardioprotective effect of exogenous S1P requires the activation of endogenous S1P via SphK.

Methods: Isolated cardiomyocytes from adult wildtype mice were exposed to 2 hours of simulated ischemia (SI) in the presence of S1P (10nM) with/without N,N-dimethylsphingosine (DMS, a SphK1 and 2 inhibitor, 10μM) or SKI (a specific SphK1 inhibitor, 15μM). Cell viability was assessed using trypan blue staining and normalised to the normoxic control.

Isolated perfused hearts from adult wildtype mice were exposed to 35 minutes of global ischemia followed by 45 minutes of reperfusion (IR) in the presence of S1P (10nM) with/without SKI (10μM). Infarct size (IS) was assessed using tripheyltetrazolium chloride staining and SphK1 activity using a specific biochemical fluorescence based assay kit. Both parameters were normalised to the IR control.

Results: In isolated cardiomyocytes, viability under normoxic conditions was 76 ± 1%. SI reduced viability to 52 ± 1% ($p < 0.001$ vs. normoxia). Pre-treatment with S1P restored the viability to 75 ± 1% ($p < 0.001$ vs. SI). The beneficial effect of S1P was partially inhibited in the presence of DMS (67 ± 4%) (ns vs. S1P) and totally abrogated with SKI pre-treatment (54 ± 2%).

Similarly, pre-treatment with S1P in isolated hearts reduced IS following IR from 50 ± 1% (IR control) to 31 ± 2% (S1P) ($p < 0.001$ vs. control). Pre-treatment with SKI abrogated the cardioprotective effect of S1P (56 ± 8%) ($p < 0.05$ vs. S1P) as well as the S1P-induced increase in SphK1 activity (from S1P: 196 ± 79 arbitrary units (AU) to SKI+S1P: 53 ± 27 AU, $p < 0.05$ vs. S1P).

Conclusions: Our data, performed in both isolated cardiomyocytes and isolated hearts subjected to an ischemia/reperfusion insult, strongly suggest that exogenous sphingosine-1-phosphate-induced cardioprotection is dependent on the activation of endogenous sphingosine-1-phosphate via sphingosine kinase 1.